

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Donald Morton
Rishab K. Gupta
David M. Euhus

Serial No.: 07/431,533

Filed: November 3, 1989

For: URINARY TUMOR ASSOCIATED
ANTIGEN, ANTIGENIC SUB-
UNITS AND METHODS OF
DETECTION

Group Art Unit: 1813

Examiner: J. Dubrule

Atty Dkt.: CADL:002/PAR

DECLARATION OF RISHAB GUPTA

Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, RISHAB GUPTA, HEREBY DECLARE AS FOLLOWS:

1. I am a joint inventor of the subject matter disclosed and claimed in the referenced application, which generally concerns Urinary Tumor Associated Antigen (UTAA), and related methods, compositions and cross-reacting antibodies.

2. I am familiar with the publication of Brown et al., U.K. Patent Application 2, 188,637, which concerns what is purported to be a tumor associated antigen designated p97. I understand that the Patent Examiner in charge of examining the referenced UTAA application has taken the position that the p97 antigen of Brown et al. is indistinguishable from UTAA.

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3. Studies have been conducted in my laboratory that conclusively demonstrate that the UTAA antigen disclosed and claimed in the referenced application is distinct from the p97 antigen of Brown et al. The present declaration is presented to make of record our studies demonstrating the uniqueness of the UTAA antigen with respect to the p97 antigen of Brown et al. as well as from a variety of other, known tumor associated antigens.

4. To rule out the possibility that UTAA corresponds to another, perhaps previously described, tumor associated antigen, a series of immunological studies were undertaken. In these studies, UTAA was subjected to a standard Western blot analysis using one of 53 murine monoclonal antibodies reactive against another putatively distinct tumor associated antigen (see Table 1). The various anti-tumor antigen antibodies used in these studies were obtained from either the listed author of the journal article describing the respective antigen, or from commercial sources, as indicated in Table 1.

5. In the Western blot studies carried out, two micrograms of the 100 kD subunit of UTAA per lane were subjected to SDS-PAGE and electroblotted to nitrocellulose membrane. After washing and blocking with 5% non-fat milk, the membrane was cut into 5mm strips. The strips were individually reacted with monoclonal antibodies at 1:100 dilution (for Mabs from ascites) or 1:25 dilution (for Mabs from hybridoma culture supernates) at 4° C.

for 12 hours. Goat anti-mouse Ig conjugated to alkaline phosphatase (Sigma Chemical Co.) at 1:500 dilution was used invariably to determine reaction of the murine monoclonal antibody. The results of these studies is set forth in Table 1 below.

6. As can be seen from Table 1, none of the 53 murine monoclonal antibodies developed by various other investigators reacted with UTAA. However, under similar conditions, monoclonal antibody AD1-40F4, at 1:500 dilution of ascites showed positive reaction with UTAA. Mab AD1-40F4 is an IgM monoclonal antibody having reactivity for UTAA, described in our referenced patent specification in Examples V, VI and VII (pages 28-31). This demonstrates that none of the monoclonal antibodies tested recognized an epitope present on UTAA.

7. It is particularly noteworthy and relevant to point out that in the above-described studies set forth in Table 1, two different anti-p97 antibodies, designated Mab 96.5 and Mab 118.1, were obtained from the Brown et al. group (Bristol-Myers), authors of the above-referenced Brown et al. publication. Neither of the anti-p97 antibodies obtained from the Brown et al. group reacted with UTAA. From this it can be said that the claimed UTAA of the present invention is distinct from the p97 of Brown et al.

8. I hereby declare that all statements of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Rishab Gupta
Rishab Gupta

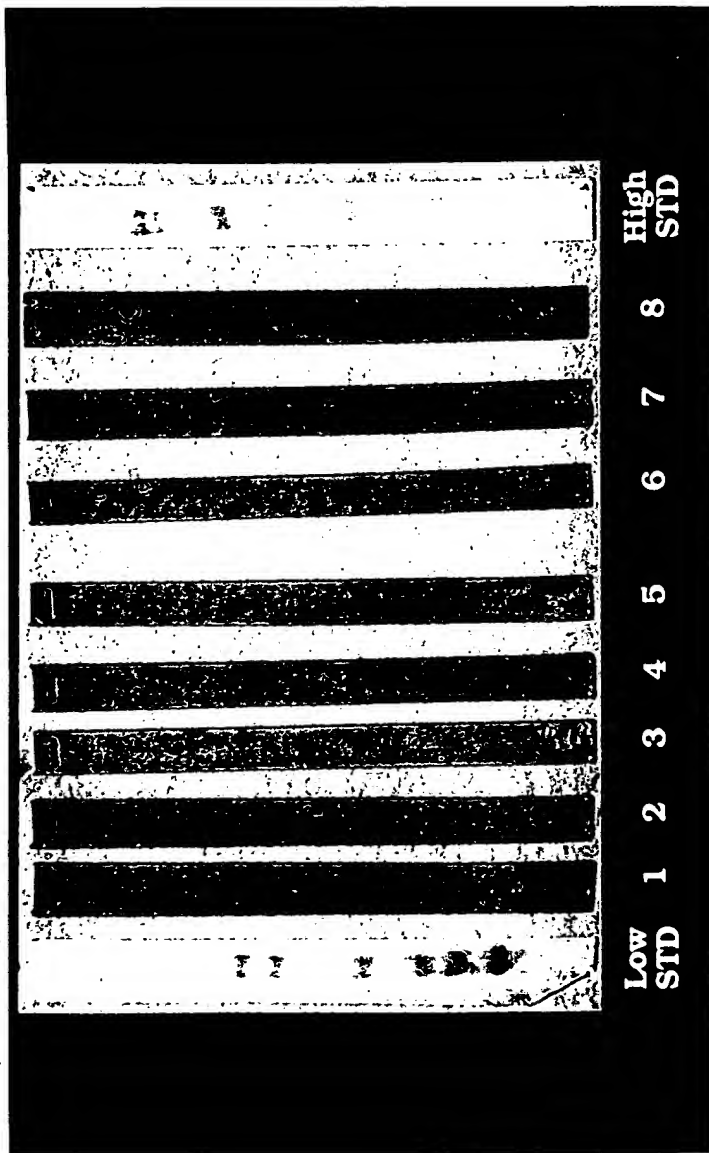
Date: June 2, 1993

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MURINE MONOCLONAL ANTIBODIES TESTED AGAINST PURIFIED U-TAA (2.0 UG PROTEIN) IN WESTERN BLOT

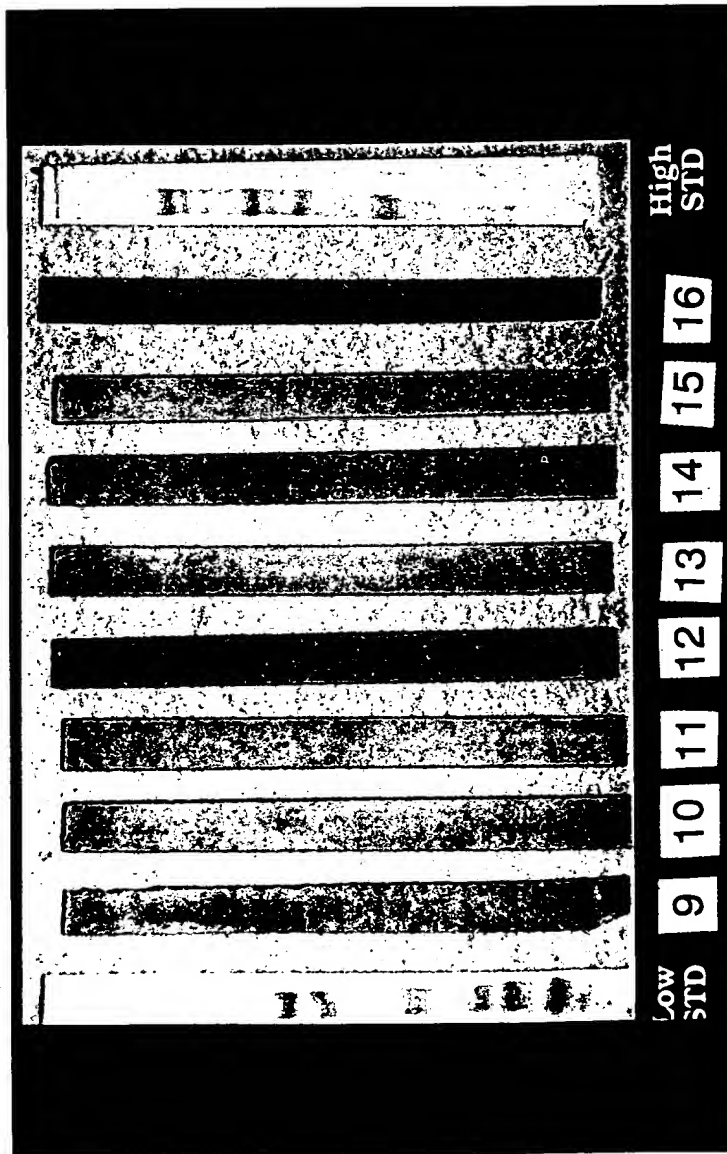
SERIAL NUMBER	MuMoAb NAME	IMMUNOGEN USED	REACTIVITY TO	DILUTION USED	W-BLOT RESULT	TYPE OF MATERIAL	ISOTYPE	VENDOR	LITERATURE REFERENCE
1	AD1-40F4	Part. purif antigen	U-TAA	1:500	POSITIVE	ASCITES	IgM	JWI	EUHUS ET AL J. CLIN. LAB. ANAL. 3:184, 1989
2	96.5	Melanoma cells	p97	1:100	NEGATIVE	PURIF. AB	IgG2a	BRISTOL-MYERS	BROWN ET AL J. IMMUNOL. 127: 539-546, 1981
3	118.1	Melanoma cells	p97	1:100	NEGATIVE	PURIF. AB	IgG2a	BRISTOL-MYERS	BROWN ET AL J. IMMUNOL. 127: 539-546, 1981
4	B5.2 (MEL-2)	SK-MEL-93	260 & >500kDa	1:100	NEGATIVE	PURIF. AB	IgG2a	SIGNET LABS.	HOUGHTON ET AL JEM 156:1755,1982
5	L101 (MEL-4)	SK-MEL-33	130 kDa	1:100	NEGATIVE	PURIF. AB	IgG2a	SIGNET LABS.	HOUGHTON ET AL JEM 156:1755,1982
6	Ta99 (MEL 5)	SK-MEL-23	75 kDa	1:100	NEGATIVE	PURIF. AB	IgG2a	SIGNET LABS.	HOUGHTON ET AL JEM 156:1755,1982
7	3G2-C6	MGH-U1 (BLADDER-CA)	92 kDa	1:100	NEGATIVE	ASCITES	IgG1	MASS GEN HOSP	YOUNG ET AL CAN RES 45:4439,1985
8	C3	MGH-U1 (BLADDER-CA)	600 kDa	1:100	NEGATIVE	ASCITES	IgG1	MASS GEN HOSP	YOUNG ET AL CAN RES 45:4439,1985



Low STD 1 2 3 4 5 6 7 8 High STD

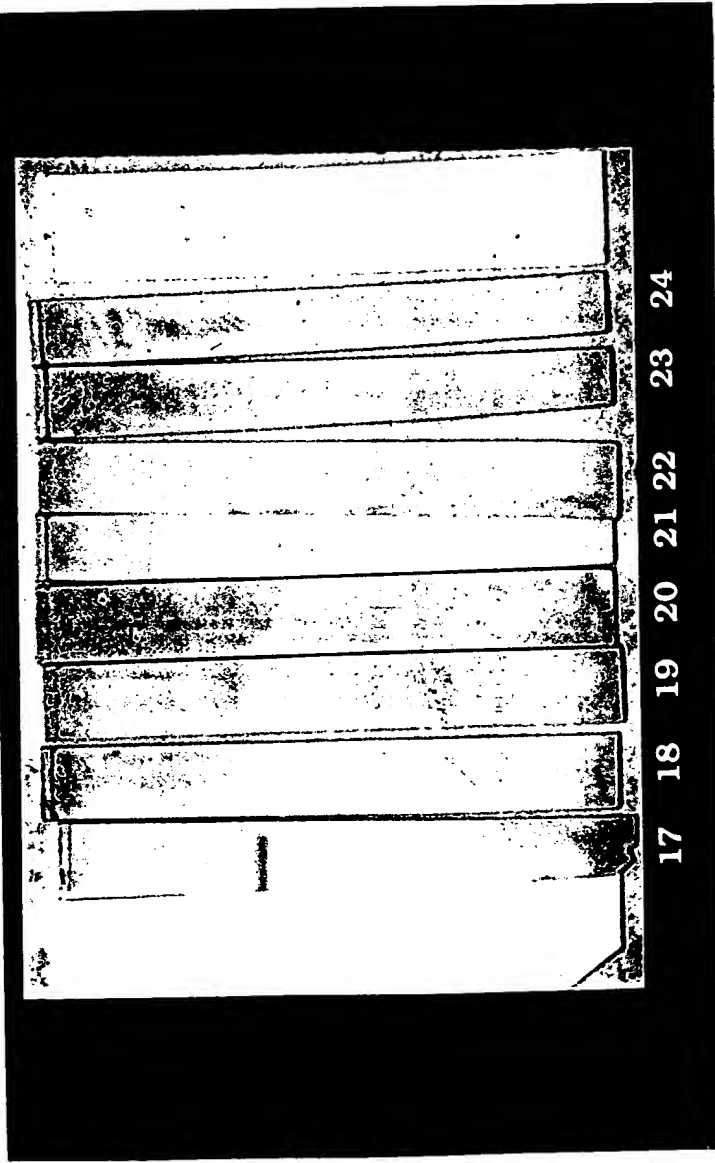
MURINE MONOCLONAL ANTIBODIES TESTED AGAINST PURIFIED U-TAA (2.0 UG PROTEIN) IN WESTERN BLOT

SERIAL NUMBER	MuMoAb NAME	IMMUNOGEN USED	REACTIVITY TO	DILUTION USED	W-BLOT RESULT	TYPE OF MATERIAL	ISOTYPE	VENDOR	LITERATURE REFERENCE
9	AD1-40F4	Part. purif	U-TAA	1:500	POSITIVE	ASCITES	IgM	JWI	EUHUS ET AL J. CLIN. LAB. ANAL. 3:184, 1989
10	G7E2	HU MEL CELLS	110 -120kDA	1:100	NEGATIVE	PURIF. AB	IgG1 KAPPA	BIODESIGN INT	CARRELS ET AL EUR. J. CA. CLIN. ONC.:24, S3-S13, 1988
11	NKI/BETEB	HU MEL CELLS	100 / 7 kDA	1:100	NEGATIVE	ASCITES	IgG2b	BIODESIGN INT	VENNEGOR ET AL AM. J. PATHO. 130, 179-192, 1988
12	PAL-M2	HU MEL CELLS	95/100 kDA	1:100	NEGATIVE	SUPERNATE	IgG1	BIODESIGN INT	RUITER ET AL J. INVEST. DERMATOL.:85, 2-6, 1985
13	NKI-C3	HU MEL CELLS	25 -110 kDA	1:100	NEGATIVE	SUPERNATE	IgG1	BIODESIGN INT	Mackie ET AL AM. J. PATHO. 37, 367-372, 1984
14	D19-61 (ME9-61)	MEL CELLS (P97)	P97	1:25	NEGATIVE	SUPERNATE	IgG2b	WISTAR	VALYI-NAGY, I.T. ET AL IN PRESS
15	403-77-4-2 (GP 120)	MEL CELLS (P120)	120/94 kDA VITRONECTIN	1:25	NEGATIVE	SUPERNATE	IgG1	WISTAR	VALYI-NAGY, I.T. ET AL IN PRESS
16	NU 6T3 (N4B)	MEL CELLS (P130/105)	130/105 kDA VITRONECTIN	1:25	NEGATIVE	SUPERNATE	IgG2a	WISTAR	MITCHELL ET AL PNAS 77: 7287, 1980



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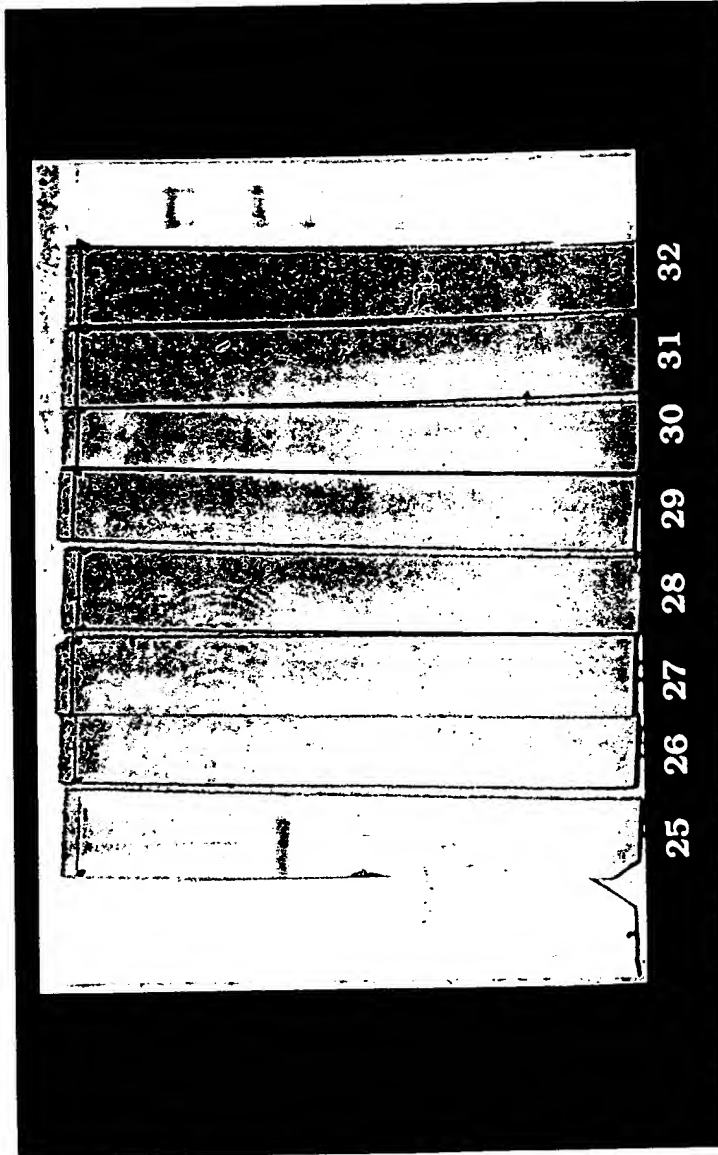
SERIAL NUMBER	MuMcAb NAME	IMMUNOGEN USED	REACTIVITY TO	DILUTION USED	W-BLOT RESULT	TYPE OF MATERIAL	ISOTYPE	VENDOR	LITERATURE REFERENCE
17	ADI-40F4	Part. purif	U-TAA	1:500	POSITIVE	ASCITES	IgM	JWI	EURUS ET AL J. CLIN. LAB. ANAL. 3:184, 1989
18	R24 (MEL-1)	SK-MEL-28	GD3	1:100	NEGATIVE	PURIF. AB	IgG3	SIGNET LAB	PUKEL, C.S. ET AL J. EXP. MED. 155:1133-1147, 1982
19	178-2-18-10 (M 77.1)	MEL CELLS	120 kDA	1:25	NEGATIVE	SUPERNATE	IgG1	WISTAR	<i>VALYI-NAGY, I.T. ET AL IN PRESS</i>
20	HM B45	MEL CELLS	30 kD	1:100	NEGATIVE	PURIF. AB	IgG1	BIOGENEX	GOWN ET AL AM. J. PATHOL 123:195-203, 1986
21	NKI-M7	MEL CELLS	150/90 kDA	1:25	NEGATIVE	SUPERNATE	IgG1	CALTAG	VRIES ET AL INT. J. CANCER:38, 465, 1986
22	PAL-M1	MEL DESCR AG	N/A	1:25	NEGATIVE	SUPERNATE	IgG1	BIODESIGN INT	RUITER ET AL J INVEST DERMATOL, 85:2, 1985
23	NKI-M6	MEL CELLS	>450/250 kD	1:25	NEGATIVE	SUPERNATE	IgG1	BIODESIGN INT	VRIES ET AL INT. J. CANCER:38, 465, 1986
24	G7A5	MEL CELLS	220-440 kD	1:100	NEGATIVE	PURIF. AB	IgG1 KAPPA	BIODESIGN INT	CARRELS ET AL EUR. J. CA. CLIN ONC. 24:S3-S13, 1988



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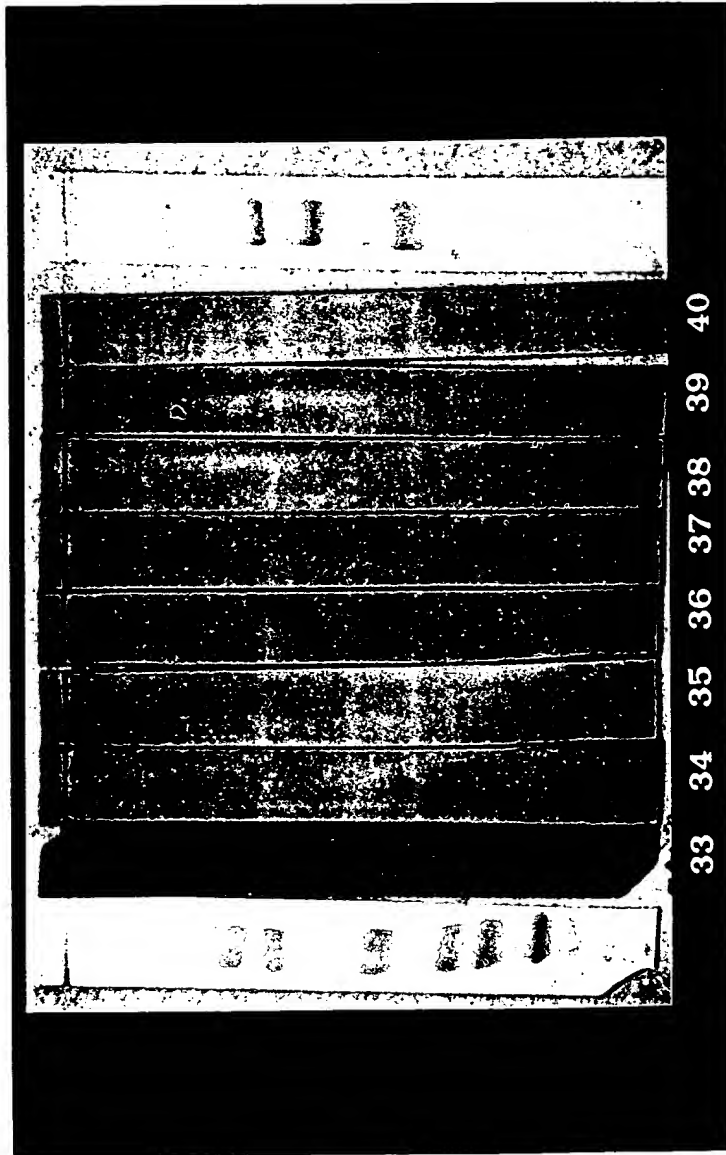
SERIAL NUMBER	MuMoAb NAME	IMMUNOGEN USED	REACTIVITY TO	DILUTION USED	W-BLOT RESULT	TYPE OF MATERIAL	ISOTYPE	VENDOR	LITERATURE REFERENCE
25	AD1-40F4	Part. purif	U-TAA	1:500	POSITIVE	ASCITES	IgM	JWI	EUHUS ET AL J. CLIN. LAB. ANAL. 3:184, 1989
26	F-11	Part. purif SPENT MEDIUM	75 / 77 & 100 kDa	1:100	NEGATIVE	ASCITES	IgG1	MARKOWITZ, REISFELD	BUMOL ET AL HYBRIDOMA 1 : 283-292 , 1982
27	9-2-27 11.98UG/ML	CELL EXTRACT MEL CELLS	240 kDa	1:100	NEGATIVE	PURIF. AB	IgG	MARKOWITZ, REISFELD	MORGAN ET AL HYBRIDOMA 1 : 27 , 1982
28	PAL-M2	N/A	95-100 kD	1:25	NEGATIVE	SUPERNATE	IgG1	MEDICA	RUITER ET AL J INVEST DERMATOL, 85:2, 1985
29	B72.3	BREAST CA	TAG-72	1:100	NEGATIVE	PURIF. AB	IgG1	SIGNET LAB	NUTI, M. ET AL INT. J. CANCER 29: 539-545, 1982
30	BT-15	SK-BR-7	80-85 kD	1:100	NEGATIVE	PURIF. AB	IgG1	SIGNET LAB	MESA-TEJADA, R. ET AL---WRONG??? AM. J. PATHOL. 130: 305-314, 1988
31	323/A3	MCF-7	43 kD	1:100	NEGATIVE	PURIF. AB	IgG1	BIOGENEX LAB	EDWARDS ET AL CANCER RES. 46:1306-1317, 1986
32	B6.2	BREAST CA	90 kD	1:100	NEGATIVE	PURIF. AB	IgG1	BIOGENEX LAB	COLCHER ET AL PROC. NATL ACAD SCI 78:3199-3203, 1981



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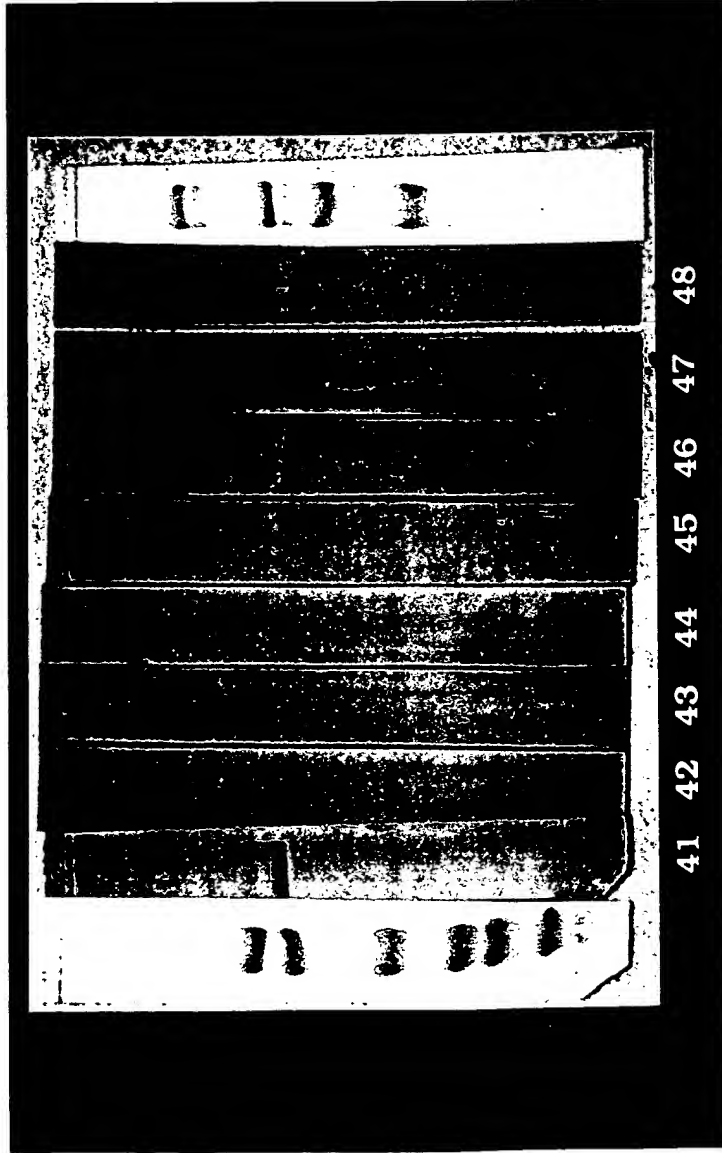
SERIAL NUMBER	MuMoAb NAME	IMMUNOGEN USED	REACTIVITY TO	DILUTION USED	W-BLOT RESULT	TYPE OF MATERIAL	ISOTYPE	VENDOR	LITERATURE REFERENCE
33	AD1-40F4	Part. purif	U-TAA	1:500	POSITIVE	ASCITES	IgM	JWI	EURUS ET AL J. CLIN. LAB. ANAL. 3:184, 1989
34	44-3A6	A549	40 kD	1:25	NEGATIVE	SUPERNATE	IgG1	AFFINITY BIOREAGENTS	RADOSEVICH ET AL CANCER RES. 45:5808-5812, 1985
35	LG-21	LUNG CA CELL	N/A	1:100	NEGATIVE	PURIF. AB	IgM	ANOGEN INC.	N/A
36	LG-26	LUNG CA CELL	N/A	1:100	NEGATIVE	PURIF. AB	IgG	ANOGEN INC.	N/A
37	TFS-4	LUNG CA CELL	N/A	1:100	NEGATIVE	ASCITES	IgG1	BIODESIGN INT	N/A
38	MOC-1	LUNG CA CELL	145 kD	1:100	NEGATIVE	PURIF. AB	IgG1	BIODESIGN INT	DE LEY L.F.M.H. ET AL EUR.J.RESPIR.DIS., SUPPL 149, V70, 1987
39	MAB455	COL-RECT CA	24 /27 kD	1:100	NEGATIVE	PURIF. AB	IgG2b	CHEMICON INT.	N/A
40	MAB425	CEA	N/A	1:100	NEGATIVE	PURIF. AB	IgG1	CHEMICON INT.	N/A



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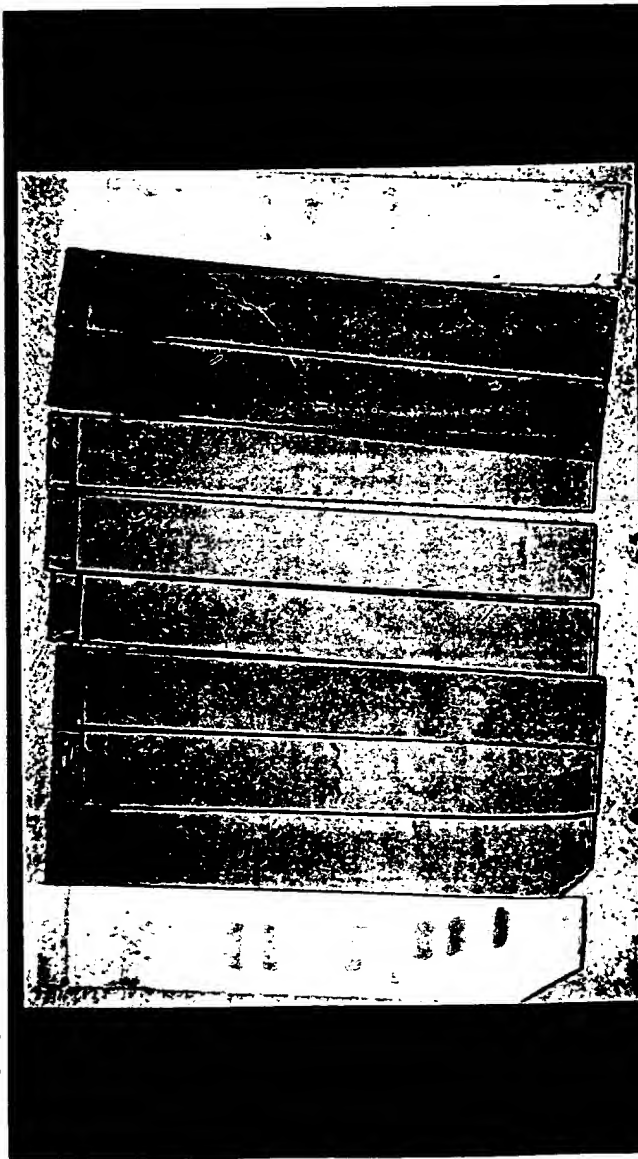
SERIAL NUMBER	MuMoAb NAME	IMMUNOGEN USED	REACTIVITY TO	DILUTION USED	W-BLOT RESULT	TYPE OF MATERIAL	ISOTYPE	VENDOR	LITERATURE REFERENCE
41	AD1-40F4	Part. purif	U-TAA	1:500	POSITIVE	ASCITES	IgM	JWI	EUHUS ET AL J. CLIN. LAB. ANAL. 3:184, 1989
42	MAB455	COL-RECT CA	24 /27 kD	1:100	NEGATIVE	PURIF. AB	IgG2b	CHEMICON INT.	N/A
43	S22 (URO-7)	SK-RC-7	115 kD	1:100	NEGATIVE	PURIF. AB	IgG1	SIGNET LAB	UEDA, R. ET AL PROC.NATL.ACAD.SCI.78:5122-5126,1981
44	YH1	PSA	N/A	1:100	NEGATIVE	PURIF. AB	IgG1	ANOGEN INC.	
45	8	PSA	35 kD	1:100	NEGATIVE	PURIF. AB	IgG	BIOGENEX LAB	WANG, M.C. ET AL INVEST UROL 17:159-163,1979
46	518-01a	POA	800-900 kD	1:100	NEGATIVE	PURIF. AB	IgG2a KAPPA	GELCO DIAGN	GELDER, F.B. ET AL CANCER RES 38:313,1978
47	DU-PAN-2	HPAF	N/A	1:100	NEGATIVE	PURIF. AB	IgM	BIOGENEX LAB	METZGAR, R.S. ET AL CANCER RES. 42: 601-608, 1982
48	512-01a	ACAA	N/A	1:100	NEGATIVE	PURIF. AB	IgG1	GELCO DIAGN	PINTO, V.B. ET AL CANCER RES 46:6520-6524,1986



41 42 43 44 45 46 47 48

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SERIAL NUMBER	MuMoAb NAME	IMMUNOGEN USED	REACTIVITY TO	DILUTION USED	W-BLOT RESULT	TYPE OF MATERIAL	ISOTYPE	VENDOR	LITERATURE REFERENCE
49	AD1-40F4	Part. purif	U-TAA	1:500	POSITIVE	ASCITES	IgM	JWI	EUHUS ET AL J. CLIN. LAB. ANAL. 3:184, 1989
50	140/S	AFP	N/A	1:100	NEGATIVE	ASCITES	IgG1 KAPPA	CHEMICON INT.	N/A
51	bcr (AB-2)	bcr	160/130 kD	1:100	NEGATIVE	PURIF. AB	IgG2a	ONCOGENE SCI	DHUT, S. ET AL ONCOGENE 3:561-566, 1988
52	115D8	MAM-6	>400 kD	1:100	NEGATIVE	ASCITES	IgG2b	CALTAG	HILKENS ET AL INT. J. CANCER, 34: 197, 1984
53	JSB-1	MDR CELLS P 170	170-180 kD	1:100	NEGATIVE	PURIF. AB	IgG1	CALTAG	SCHUPER, R.J. ET AL INT. J. CANCER, 42: 389-394, 1988
54	OV 632	OVAR. CA	N/A	1:100	NEGATIVE	PURIF. AB	IgG2b	CALTAG	FLEUREN, G.J. ET AL VIRCHOWS ARCHIV A, 1987 IN PRESS.
55	RC 38	RENAL CA	N/A	1:100	NEGATIVE	SUPERNATE	IgG1	CALTAG	OOSTERWIJK, E. ET AL AM. J. PATHOL, 123:301, 1986
56	123C3 (CD 56)	SMALL CELL CARC.	29/150 kD	1:100	NEGATIVE	ASCITES	IgG1	CALTAG	N/A



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SERIAL NUMBER	MuMoAb NAME	IMMUNOGEN USED	REACTIVITY TO	DILUTION USED	W-BLOT RESULT	TYPE OF MATERIAL	ISOTYPE	VENDOR	LITERATURE REFERENCE
57	AD1-40F4	Part. purif	U-TAA	1:500	POSITIVE	ASCITES	IgM	JWI	EUHUS ET AL J. CLIN. LAB. ANAL. 3:184, 1989
58	27C10 (4/12/93)	MEL CELL	87 kDA	1:25	NEGATIVE	SUPERNATES	IgG	S.K. LIAO	LIAO, S.K. ET AL UNPUBLISHED
59	140.240A (4/11/93)	MEL CELL	87 kDA	1:25	NEGATIVE	SUPERNATES	IgG2a	S.K. LIAO	LIAO, S.K. ET AL MOL. IMMUNOL. 24: 1-9, 1987

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